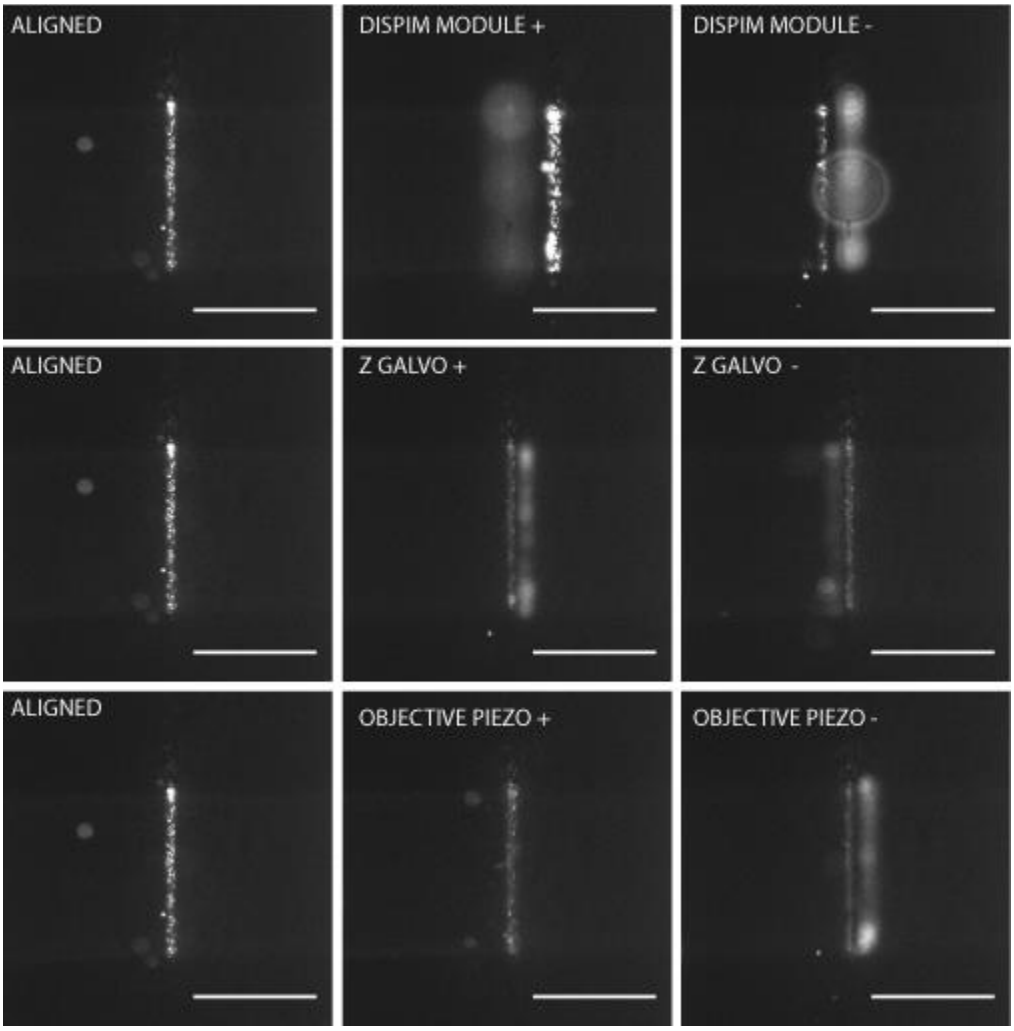
Misalignment in diSPIM components diagnosed through arm A camera



All images show both epifluorescence signal detected in arm A when illuminating with the sheet generated by arm A, and fluorescence signal detected in arm A when illuminating with the sheet generated by arm B. When all system components are aligned, the two fluorescence signals should overlap, resulting in a crisp image of the fluorescent beads (left column). The other panels show various misalignments, causing a blurry fluorescence signal resulting from the sheet generated by arm B (the epifluorescence signal stays in focus): 'DISPIM MODULE +', the diSPIM module has been moved 12 μm above the optimal position; 'DISPIM MODULE -', the diSPIM module has been moved 10 μm below the optimal position; 'Z GALVO +', arm B's Z Galvo has been positioned 0.08 V (sheet has moved 6.5 μm) above the optimal value; 'Z GALVO -', arm B's Z Galvo has been positioned 0.1 V (sheet has moved 8 μm) below the optimal value; 'OBJECTIVE PIEZO +', arm A's objective piezo has been positioned 1 V (plane of detection has moved 20 μm) above the optimal value; 'OBJECTIVE PIEZO -', arm A's

objective piezo has been positioned 1 V (plane of detection has moved 20 μm) below the optimal value. The sample is a coverslip coated with yellow-green fluorescent beads. Scalebars: 50 μm.